Current Biology, Vol. 14, R56–R58, January 20, 2004, ©2004 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.cub.2003.12.043

Recombination: Holliday Junction Resolution and Crossover Formation

Dispatch

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The heterodimeric nuclease Mus81–Eme1 has been proposed to be a Holliday junction resolvase and has now been found to be responsible for nearly all meiotic crossovers in fission yeast. The intriguing substrate preference of this enzyme for nicked Holliday junctions opens the possibility that crossover formation may not always involve double Holliday junctions.

Holliday junctions have been considered a central intermediate in homologous recombination ever since Robin Holliday proposed their existence and suggested that crossovers result from their resolution [1]. While recombination models have evolved since that time, the Holliday junction — more precisely the double Holliday junction [2] — is still held as the critical intermediate in crossover formation [3] (Figure 1). In *Escherichia coli*, RuvC and RusA were identified as Holliday junction resolvases [4], but the equivalent resolvases of eukaryotes have proved elusive [4,5].

The heterodimeric enzyme Mus81–Eme1, a DNA structure-specific endonuclease of the XPF family [5–7], has been proposed to be a Holliday junction resolvase in fission yeast and human cells [8,9]. Three papers [10–12] have now reported compelling evidence that Mus81–Eme1 is responsible for the vast majority of crossovers in fission yeast meiosis, and that the Mus81–Eme1 endonuclease activity displays an intriguing substrates preference for nicked Holliday junctions and D-loops. The groups propose two different models for how Mus81–Eme1 achieves crossover formation: by classical resolution of double Holliday junctions (Figure 1) [11,12]; or by a novel pathway involving consecutive cleavage of D-loops and nicked Holliday junctions, without involving double Holliday junctions (Figure 2) [10].

Genetic and biochemical data led Russell and colleagues [8,9] to propose that Mus81–Eme1 is the principal Holliday junction resolvase of fission yeast. The primary genetic observation was that *mus81* mutants undergo abortive recombination leading to a failure to segregate the DNA mass and a spore viability that is lower than expected for random segregation of the three fission yeast chromosomes. These defects were almost completely reversed by the expression of the bacterial Holliday junction resolvase RusA [8,10].

Two of the new papers [10,12] report compelling recombination data showing that meiotic crossover frequencies are reduced 20–100-fold in the surviving spores of a *mus81* meiosis. The combined data span six intervals on all three fission yeast chromosomes, covering over 900 cM or about half of the genome, and show that the effect is genome-wide. Importantly, both studies show that meiotic intragenic recombination, which proceeds by conversion and not by crossover, is normal or even elevated in *mus81* mutants. While more than half of the intragenic recombinants (convertants) were associated with a crossover in wild-type cells, convertants in *mus81* cells showed not a single associated crossover [10]. As for the spore formation and the spore viability defects, the crossover deficiency of *mus81* mutants was significantly suppressed by expression of the RusA resolvase [12].

Only the minority fraction of surviving spores could be analyzed in these studies, but from the results of the RusA experiments, it is inferred that a general junction resolution defect led to meiotic lethality. Smith *et al.* [12] and Gaillard *et al.* [11] propose that Mus81–Eme1 resolves Holliday junctions. In the context of the recombination model depicted in Figure 1, Mus81–Eme1 would act in the double-strand break repair pathway to generate crossovers by resolution of double Holliday junctions (Figure 1, step 5→5a). In the absence of Mus81–Eme1, conversion-type recombinants could still be generated by resolving double Holliday junctions using topological means [5] or by synthesis-dependent strand annealing (Figure 1). Unfortunately, direct physical analysis of recombination intermediates is not yet possible in fission yeast meiosis, leaving the exact physical nature of the meiotic recombination intermediates that accumulate in *mus81* cells undetermined.

A wealth of information is available on the *in vitro* substrate preference of Mus81-containing endonucleases [5,8–11,13,14]. The experiments have been performed with either the highly purified recombinant enzyme made in bacteria, or the partially purified endogenous enzyme from the cognate host. An earlier controversy over the substrate preference of Mus81-containing endonucleases appears to have been resolved (reviewed in [5]), and the new reports by Gaillard *et al.* [11] and Osman *et al.* [10] provide further insights. Both studies show that recombinant and endogenous Mus81–Eme1 from fission yeast, as well as recombinant budding yeast Mus81–Mms4, prefer nicked Holliday junctions over any other substrate, including 3′-flaps or intact Holliday junctions. Gaillard *et al.* [11] conclude that Mus81–Eme1 operates by a nick and counternick mechanism, similar to that of RuvC and other resolvases which also display a large enhancement of second-strand cleavage after a rate-limiting first-strand cleavage [4].

Recombinant and endogenous Mus81-containing enzymes differ in their activity on intact Holliday junctions: only the endogenous enzymes from fission and human cells, and not the recombinant enzymes, show appreciable cutting of intact Holliday junctions [8–11,15]. This suggests that an endogenous post-translational modification and/or a cofactor is missing

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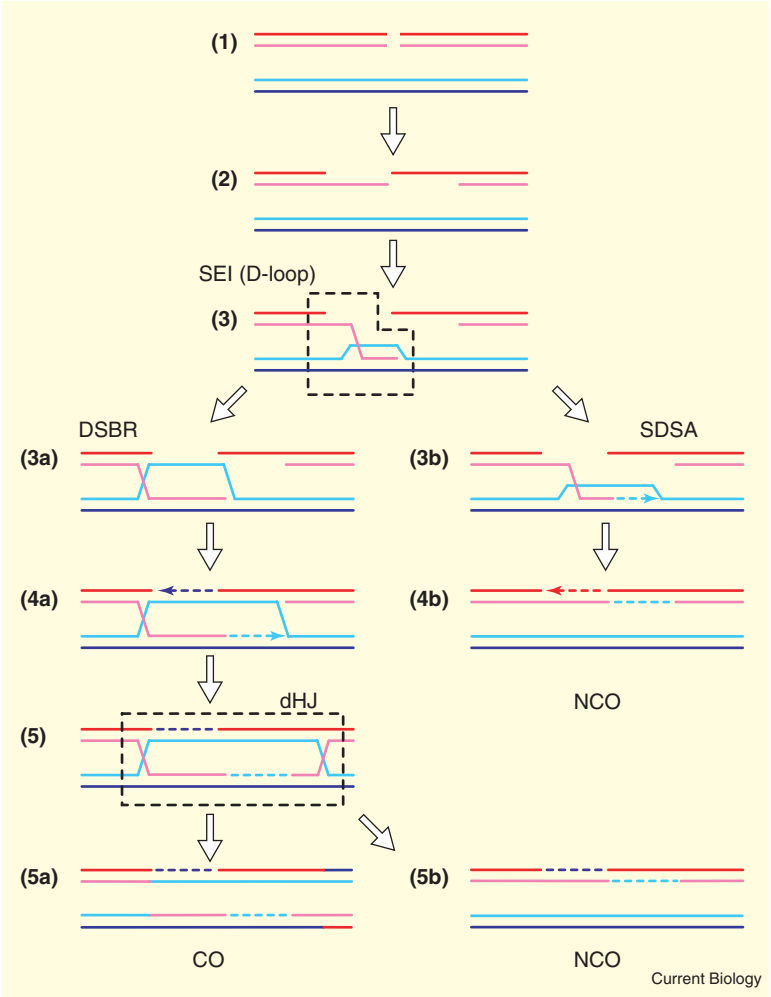

Figure 1. Double-strand-break repair (DSBR) and synthesis-dependent strand annealing (SDSA) models for meiotic recombination.

Meiotic recombination is initiated by Spo11-generated double-stranded DNA breaks (DSBs) (step 1). After DSB processing by the Mre11–Rad50–Xrs2 complex and other enzymes (step 2), RPA, Rad52 and Rad55–Rad57 orchestrate formation of the Rad51 nucleoprotein filament capable of homology search and DNA strand invasion (step 3). Rad54 augments Rad51-mediated recombination in D-loop formation and is also thought to allow access of DNA polymerases to the invading 3′-OH by displacing Rad51 from the product heteroduplex DNA (steps 3b, 4a). The D-loop created by single-end invasion (SEI) may enter the DSBR pathway (step 3a) and form a double Holliday junction (dHJ; steps 4a, 5), which can be resolved to crossover (CO; step 5a) and non-crossover (NCO; step 5b) products. Resolution to CO requires a symmetric cleavage of both Holliday junctions in opposite orientations by Holliday junction resolvase. Resolution to NCOs can also be achieved by the resolvase (cleavage of both junctions in the same orientation) and by collapsing the dHJ to a hemi-catenane followed by resolution involving a type I topoisomerase activity. Alternatively, the D-loop enters the SDSA pathway (step 3b). After extension by DNA polymerase, the invading strand retreats to reanneal with the single-stranded DNA tail that did not form a D-loop (step 4b). In its simplest version, SDSA leads only to NCO products (as shown). More complex versions of SDSA involve dHJ formation, which can be resolved by a Holliday junction resolvase to CO and NCO products [3].

in the recombinant preparations. Such a modification or cofactor was proposed to affect the first nick during Holliday junction cleavage, as the initial nick was found to be the rate limiting step in Holliday junction resolution by Mus81–Eme1 [11].

How do the fission yeast data mesh with those on budding yeast meiosis? In some budding yeast strains, *mus81* (*mms4*) mutants are less affected in meiosis than their fission yeast counterparts, and in the surviving spores crossover formation is only slightly reduced [6,16]. This is not surprising, as these two yeasts differ greatly in meiosis. Budding yeast, like most eukaryotes, develops a synaptonemal complex during meiotic prophase, which is believed to be important for meiosis, in particular for crossover formation and crossover interference [3]. Fission yeast does not develop a synaptonemal complex during meiosis and does not show crossover (chiasma) interference. Interestingly, crossover interference is much lower on short chromosomes in budding yeast, and here the contribution of Mus81 to crossovers is the greatest [16]. Thus, while fission yeast relies heavily on Mus81 in meiotic crossover formation, budding yeast employs multiple pathways, including Mus81–Mms for the crossover pathway without interference and other, yet to be identified, resolvase(s) in the crossover pathway with interference.

What is the *in vivo* substrate of Mus81–Eme1/Mms4? Some answers are already available. Gaillard *et al.* [11] found that X-shaped molecules — Holliday junctions — accumulated in the rDNA of *mus81* mutants genetically sensitized by a mutation in DNA polymerase α (encoded by *pol1*). This could explain the negative synergy between mutations in *mus81* and *pol1* [7], and supports the view that Mus81–Eme1 resolves Holliday junctions *in vivo* [11,12]. Detailed genetic and physical analyses in budding yeast meiosis provided compelling evidence that the double Holliday junction is the critical intermediate in forming crossovers that display interference [17,18]. A similar analysis in *mms4* cells did not show an increase in double Holliday junctions, as expected from a resolution defect, but rather a decrease [16]. Thus, double Holliday junctions appear as an unlikely *in vivo* substrate for Mus81–Mms4 in budding yeast meiosis.

Could the *in vivo* substrates for Mus81–Eme1/Mms4 be different in fission and budding yeast? This is possible! In *Drosophila* meiotic crossovers largely depend on *mei-9*, which encodes the fly XPF homolog [19]. In both yeasts, a contribution of the XPF homologs

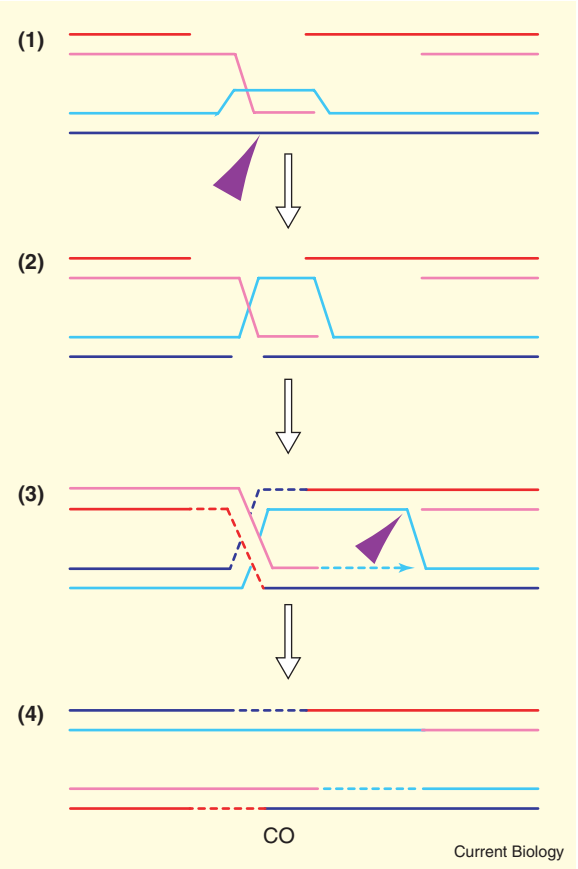

Figure 2. A model for crossovers formation without resolution of double Holliday junctions.

After single-end invasion, Mus81–Eme1 cleaves the D-loop, which should topologically stabilize single-end invasion. Second-end capture and DNA synthesis produces a nicked Holliday junction subject to a second, possibly independent, cleavage by Mus81–Eme1. The potential Mus81–Eme1/Mms4 cleavage sites are indicated by arrowheads. Processing of the cleavage products to accommodate possible flaps and ligation will always lead to crossovers. Osman *et al.* [10] propose this alternative crossover model based on the *in vitro* substrate specificity of Mus81–Eme1. This model was independently [5] derived from the proposed function of XPF–ERCC1 in gene targeting [20]. XPF–ERCC1 is related to Mus81–Eme1/Mms4, the enzymes having a similar subunit structure and sequence homology in their active sites [5].

to meiotic crossover has been all but excluded [3,5]. Note that XPF and Mus81 are closely related endonuclease subunits (see Figure 2 legend).

Might Mus81–Mms4 have other *in vivo* substrates than Holliday junctions? Mus81–Eme1 specifically cleaves a D-loop [10] (Figure 2), which resembles a nicked Holliday junction, its preferred substrate [10,11]. Such a cleavage is expected to stabilize strand invasion. Thus, the expected mutant phenotype would be a decrease in single-end invasions (D-loops). Exactly this was found in budding yeast *mms4* mutants [16]. From the Mus81–Eme1 substrate preference for D-loops and nicked Holliday junctions, Osman *et al.* [10] propose an interesting model that exclusively generates crossover without the involvement of double Holliday junctions [10] (Figure 2). This model is a radical deviation from the crossover models that involve resolution of single and double Holliday junctions (Figure 1). Is this how crossovers are generated outside the context of a synaptonemal complex? The study of Mus81-containing endonucleases has brought fresh ideas and exciting data to the recombination field. This will help to solve one of the fundamental questions in genetics: how are crossovers generated?

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